10 d) assaying said culture medium for said secreted cellular antigens characteristic of said tissue, cells, ascites, or effusion fluid, and indicative of a disease state or lack thereof , and detecting said secreted cellular antigens.

--23. The method according to claim 1 wherein said cohesive multicellular particulates are of a particle size of roughly 1 mm^3.--

REMARKS

Claims 1, 4, 5, 15 and 16 have been amended. Claim 23 has been added. Claims 1, 3-7, and 9-22 remain in the application. Reexamination and reconsideration of the application as amended is requested.

The Examiner has provisionally rejected claims 1, 3-7 and 9-22 under the doctrine of obviousness double patenting. By means of the undersigned, Applicant herewith files a Terminal Disclaimer in compliance with 37 CFR 1.321(c) to limit the term of the patent issuing from the current application to the term of United States Patent No. 5,728,541 and to the term of any patent issuing from U.S. Patent Application Serial No. 09/095,993. Besides these, there are no other related pending applications.

The Examiner has rejected claims 1, 3-7 and 9-22 under 35 U.S.C. § 102(b) for asserted anticipation by each of U.S. Patent No. 5,242,806 to Yen-Maguire, U.S. Patent No. 4,423,145 to Stampfer, U.S. Patent No. 5,270,172 to Morgan, and U.S. Patent

No. 4,937,187 to Rotman. However, particularly in view of the claim amendments made herewith, none of these references teaches the use of tissue particles of the size claimed in the current application. Support for the particle sizes as amended is found on page 16, line 6 of the present specification. A detailed explanation follows.

The Examiner maintains that Yen-Maguire teaches measuring the responsiveness of multiple cell populations rather than single cell populations, eliminates the requirement for single cell suspensions, and states that, even if cells are seeded as aggregates, the cells will spread out. Yen-Maguire does refer to methods in which multiple-cell populations are used in an assay (column 3, line 8), the strict requirement for single-cell suspensions is eliminated (column 3, line 58), and cells seeded as aggregates spread out (column 10, line 23). The experimental teachings of Yen-Maguire, however, are in an entirely different direction from the present invention. The Yen-Maguire procedure begins with the mincing of a specimen into pieces less than 1 mm in size (column 12, line 60). Though all pieces are less than 1 mm in size, the larger tissue pieces are avoided (column 12, line 62). Rinsing of the tissue is used to produce an adequate cell number (column 12, line 65), so the method relies on single cells or groups of cells that can be removed from a tissue sample by rinsing. If this procedure produces an inadequate cell count, the use of a tissue sieve is recommended; the desired outcome is the dispersion of cells (column 13, line 8). If the tissue sieve does not produce a cell dispersion, the use of digestive enzymes to free single cells is

recommended (column 13, line 10). The method of Yen-Maguire focuses on the production of disaggregated cells, instead of on the use of multicellular particulates; as in the present application.

Though the method of Yen-Maguire may include the use of aggregates it is, therefore, clear in context that these aggregates are much smaller than the "about 0.25 mm^3 to 1.5 mm^3" multicellular particulates of the present application. Small cell aggregates are characterized (column 3, line 33) as containing fewer than 50 to 100 cells. Most cells are between 10 and 30 microns (or micrometers) in diameter; cells in which mitosis has been suppressed may approach 50 microns in diameter. One hundred cells with a diameter of 50 microns, arranged in a loosely-packed body-centered cubic (bcc) configuration would occupy a volume of 5.0×10^{-4} mm^3, a volume far smaller than the lower particle size limit of 0.25 mm^3 in claim 1. (Cell size information was obtained from the Robert H. Lurie Comprehensive Cancer Center of Northwestern University at http://www.cancergenetics.org/basis.htm. Information on giant cells produced from colorectal tumor cells with a diameter approaching 50 microns was obtained from the Ph.D. thesis of Tim Bracey (University of Bristol) and a related publication in *Clinical Cancer Research* 3, 1371-1381, August 1997). For these reasons, it is believed that claims 1, 3-7 and 9-22 are not anticipated by Yen-Maguire.

The Examiner maintains that Stampfer teaches the culturing of clumps of cells obtained from a biopsy, and the determination with varying concentrations of adriamycin

sensitivity to specimens. However, Stampfer teaches the use of clumps of cells obtained by enzymatic digestion. The cohesive multicellular particulates used in the present invention are obtained through mechanical separation. The claims of the present application, as revised, are written in "consisting essentially of" terminology, which excludes disaggregating the specimen as taught by Stampfer.

Stampfer does not state the number of cells contained in a clump, but does teach the use of polyester and nylon uniform pore-size filters for the removal of undesired cellular components from the suspension containing cells and cell clumps (column. 3, line 46). Commercially available polyester and nylon membrane filters are available with pore sizes ranging from 0.1 micron to 20 microns. The undersigned is aware, for example, of product literature for polyester and nylon membrane filters produced by Pall, Inc. and by Micron Separations, Inc., listing pore sizes. Information copies are submitted herewith for the consideration of the Examiner. Applicant is prepared to submit this information more formally, by means of an expert's declaration, if necessary. Moreover, as cited above, most cell sizes are between 10 and 30 microns in diameter. The smallest cells in this range have half the diameter of the largest pore in the type of membrane filter used, so that a clump of cells passing through a pore would have to present a cross-section of, at most, two or three cells. Because Stampfer uses polyester or nylon membrane filters to remove undesired cellular components from suspension, the cells and cell clumps must be small enough to pass through the filters. The cells and cell clumps, as a

result, have diameters of 20 microns at most. This is far smaller than the 0.8 mm diameter of a sphere having a volume of 0.25 mm^3, the lower limit for a multicellular particulate in claim 1. For these reasons, it is believed that claims 1, 3-7 and 9-22 are not anticipated by Stampfer.

The Examiner maintains that Morgan discloses the mincing into fragments and culturing of cancer tissue and the assaying of chemotherapeutic drugs and doses. Although the Morgan patent contains a description of plating tissue particulates into one or more plates and using the plated samples for chemosensitivity assays, the 0.1 mm particulates Morgan discloses are significantly smaller than the multicellular particulates of the present claims, namely, about 0.25 mm^3 to about 1.5 mm^3. For this reason, it is believed that claims 1, 3-7 and 9-22 are not anticipated by Morgan.

The Examiner maintains that Rotman discloses forming clumps of cells from tumor biopsies, establishing a cell culture, exposing the cell culture to a therapeutic agent, and determining the sensitivity of the cells to the agent. However, the fragments of cells disclosed by Rotman are derived from shearing or digestion of 1.0 mm^3 pieces. The mechanical, enzymatic or combined treatments lead to cellular fragments of a far smaller size than those of the present invention, preferably from about 50 to about 5000 cells (column 4, line 24). The cell clumps produced according to the Rotman technique are small enough to produce a suspension (column 4, line 26). Five thousand cells with a diameter of 50 microns, arranged in a loosely-packed body-centered cubic (bcc) configuration would occupy a volume of

2.5×10^{-2} mm^3, a volume smaller by an order of magnitude than the lower limit of 0.25 mm^3 in claim 1. For these reasons, it is believed that claims 1, 3-7 and 9-22 are not anticipated by Rotman.

The Examiner has rejected claims 1, 3-7 and 9-22 under 35 U.S.C. § 112, first paragraph, for asserted nonenablement. Specifically, the Examiner alleges that the specification does not instruct a person skilled in the art how to perform the wound healing and gene therapy assays. The Examiner also alleges that the specification does not instruct a person skilled in the art how to assess chemosensitivity of non-malignant cells, identify chemosensitivity of cells, nor identify any secreted cellular antigens produced by cells.

In view of the skill of the art, and in context, these claims can be seen to be enabled. Procedures for the evaluation of chemotherapeutic agents are set out in detail beginning on page 6 of the specification. On page 12, line 11, the specification includes a description of the analogous procedures for the assay of cell growth promoters and how chemotherapeutic agents can be used in other analyses. Although chemotherapeutic agents are most commonly used for the treatment of malignant tumors, they are not restricted to this use. They are also used for other purposes, such as immunosuppression. The specification also provides a context for the evaluation of any active agent producing an effect on the growth of cells according to the method of the present invention (page 12, line 26) and a summary of the method to be used (page 12, line 35). Techniques for the detection of markers, factors and biological response modifiers

are described on page 13, line 25 of the specification, and Example 6 of the specification (page 23, line 5) provides enablement for the assay of a biological response modifier. The present invention is directed to the screening of known active agents, not their discovery; no undue experimentation is required for the use of the invention with an already known wound healing or gene therapy agent. Accordingly, claims 10 and 17 are enabled by the specification and by the practical reality that the claimed method is practiced with known therapeutic agents.

Chemotherapeutic agents are most commonly used for the treatment of malignant tumors, but they are not restricted to this use. They are also used for other purposes, such as immunosuppression. It is believed that the use of the term "chemotherapeutic agent" in claim 9 does not imply that a malignancy is being treated. Radiation therapy is widely used for the treatment of malignant tumors, but it is not restricted to this use. It is also used for other purposes, such as immunosuppression. It is believed that the use of the term "radiation therapy" in claim 11 does not imply that a malignancy is being treated. The undersigned is aware, for example, of a *New England Journal of Medicine* editorial of August 8, 1996 describing the use of a chemotherapeutic drug for the treatment of sickle cell disease, and a document from the Tulane Bone Marrow Transplant Program describing the use of both chemotherapy and radiation therapy for the elimination of immune cells in the course of bone marrow or stem cell transplantation. Information copies are submitted herewith for the consideration of the Examiner. Applicant is prepared to submit this information more

formally, by means of an expert's declaration, if necessary.

The Examiner has rejected claims 1, 3-7, and 9-22 under 35 U.S.C. § 112, second paragraph, for asserted indefiniteness. Claim 1 has been amended to make consistent reference to a "plurality of segregated sites." Claim 1 now provides a consistent antecedent basis for the use of the term in claim 3. Claim 4 has been amended herewith to make reference to an "agent". Support for the amended terminology is found on page 5, line 33. Claim 4 has been amended to clarify that each site is exposed to a single agent.

Claim 15 has been amended to include a step to accomplish the preamble. Support for the amendment is found on page 12, line 4 of the specification.

Claim 16 has been amended to include a step to accomplish the preamble. Support for the amendment is found on page 13, line 25 of the specification.

Claim 16 has been amended to make consistent reference to cohesive multicellular particulates. Support for the amended terminology is found on page 12, line 35 of the specification.

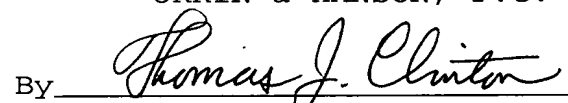
Finally, by insertion of the new transitional phrase, "consisting essentially of" in claim 1, applicant specifies that further subdivision of the tissue is not embraced by the present claims.

The methods described in the references cited by the Examiner rely on the use of digestion to reduce the size of cell masses or the mechanical subdivision of cell masses to facilitate the formation of a suspension. The formation of the multicellular particulates used in the present invention requires

neither of these procedures and is thus distinguished from the methods described in these references by recitation of dimensional size. The variations of the present invention involving cell growth promotion involve the same procedures as the evaluation of known chemotherapeutic agents for other purposes, such as cancer treatment or immune system suppression. Consequently, the specification provides sufficient guidance for the use of these variations. Therefore, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the rejections is requested. Allowance of claims 1, 3-7 and 9-22 is respectfully requested.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By 

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